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Renal brush border membrane Na/P_i-cotransport: Molecular aspects in PTH-dependent and dietary regulation

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Renal brush border membrane Na/P_i-cotransport: Molecular aspects in PTH-dependent and dietary regulation. Inorganic phosphate (P_i) is reabsorbed in renal proximal tubules in a sodium (Na)-dependent manner involving brush border Na/P_i-cotransporter(s). Regulation of renal P_i-reabsorption, such as by parathyroid hormone (PTH) and/or by dietary P_i-deprivation, involves alterations in the rate of Na/P_i-cotransport. Two structurally different Na/P_i-cotransporters have been identified: type I-transporter and type II-transporter. The related mRNAs and proteins are located in the proximal tubule and in the brush border membrane. In heterologous expression systems type I and type II Na/P_i-cotransporters mediate Na/P_i-cotransport. Characterization of the transport properties suggested that the type II transporter is 'responsible' for brush border membrane Na/P_i-cotransport (as observed in isolated vesicles). Administration of PTH to rats resulted in an inhibition of brush border membrane Na/P_i-cotransport (vesicles) and in a reduced brush border membrane content of the type II transporter. Feeding low P_i-diets resulted in an up-regulation of Na/P_i-cotransport (vesicles) and of type II transporter content; only after a prolonged exposure to low P_i-diets (more than 4 hr) was an increase in specific mRNA content observed. Refeeding high P_i diets had the opposite effects on Na/P_i-cotransport activity and on type II transporter protein. It is currently the task of future experiments to define the specific mechanisms leading to protein-synthesis-independent (PTH, acute P_i-deprivation, P_i-refeeding) and to protein-synthesis-dependent (prolonged P_i-deprivation) regulation of the type II Na/P_i-cotransporter.

The kidney plays a key role in controlling P_i-homeostasis, mainly by altering proximal tubular reabsorption [1–4]. *In vivo* and *in vitro* microperfusion studies and experiments on isolated brush border membrane vesicles have previously documented that brush border membrane sodium-dependent phosphate transport mechanisms [Na/P_i-cotransporter(s)] are the rate limiting steps in P_i-reabsorption [4–7]. These 'apical' transport steps are also participating in physiological (and pathophysiological) alterations in renal P_i handling [3–7]. For example, parathyroid hormone (PTH)-dependent control and dietary intake-dependent alterations of urinary P_i excretion are due to alterations in the rates of brush border membrane Na/P_i-cotransport (see below). Similarly, the renal P_i leak in X-linked hypophosphatemia is related to a reduction in renal Na/P_i cotransport activity [8]. The recent molecular identification, by expression cloning techniques, of renal brush border membrane Na/P_i-cotransporters not only permitted an identification of the molecular structure of these transporters [9–11], but also gave more information about physiological and pathophysiological alterations of these transporters at the molecular level. In this brief overview we shall first describe the molecular structure and 'cell localization' of the renal brush border Na/P_i-cotransporter(s) and then discuss some aspects of physiological/pathophysiological regulation. For the latter aspect we will concentrate on PTH and dietary P_i-intake as well as on X-linked hypophosphatemia.

'Functional' and 'structural' aspects of renal brush border Na/P_i-cotransporters

Two entirely different renal brush border Na/P_i-cotransporters (type I and type II Na/P_i-cotransporter [10]) have been identified by expression cloning techniques [9, 11–20]. When expressed in *Xenopus laevis* oocytes or in MDCK cells both transporters mediate Na-dependent P_i-uptake (Na/P_i-cotransport); the type II transporter was also functionally expressed in insect sf-9 cells [9, 11, 14–16, 18–24]. Extensive characterization of type I and type II mediated transport into *Xenopus laevis* oocytes, by either tracer techniques or by electrophysiological techniques, showed overall characteristics compatible with the properties previously obtained in studies on isolated vesicles and/or from intact tissue studies only for the type II transporter [5–7, 9–11, 15, 21, 25, 26]. In brief, the type II transporter accepts both inorganic phosphate and arsenate as substrates, and most likely co-transports 3 Na-ions. Furthermore, it shows a characteristic pH-dependence, that is, higher rates at more alkaline pH-values and at physiological P_i- and Na-concentrations, which is most likely explained by a competition of protons with Na interaction rather than by preferential transport of divalent P_i [5, 9, 15, 21, 25, 26]. The type I transporter seems also to transport P_i in a Na-dependent manner, but apparently also functions as an anion channel accepting chloride and several organic anions such as probenecid and benzyl-penicillin **(Note added in proof).**

The type I Na/P_i-cotransporter is around 465 and type II cotransporter 635 amino acids long [9–11]. In Western blots using isolated brush border membrane vesicles the type I transporter has an apparent molecular weight of 60 to 65 kDa and the type II transporter of 80 to 90 kDa [27, 28]. In accordance with *in vitro* translation experiments (plus/minus microsomes, plus/minus endoglycosyclase treatments) we concluded that both transporters

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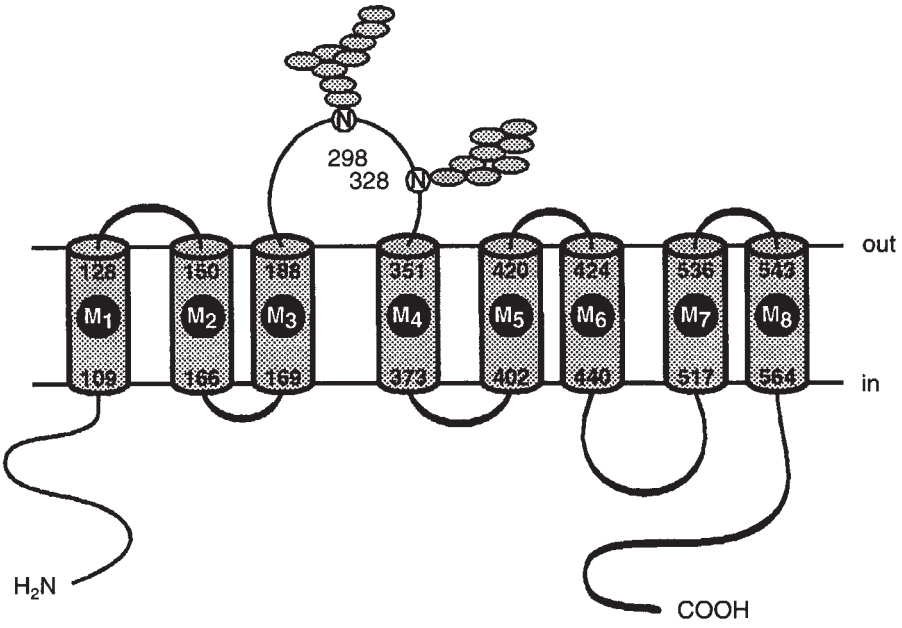

Fig. 1. *Secondary structure prediction for the type II Na/P_i-cotransporter.* According to hydropathy analysis the transport protein spans at least eight times the brush border membrane. The transporter is N-glycosylated at the 2 positions indicated (for further details [9, 10, 29]).

(type I and type II) are glycosylated [9–11]. Mutagenesis experiments on the type II transporter provided evidence for the preferential glycosylation at two sites (Fig. 1) [10, 29]. Hydropathy analysis predicted that the type II cotransporter spans at least eight times the membrane [9, 10]. With the available knowledge on the glycosylation sites and the most likely cytoplasmic orientation of both the carboxy- and NH_2-termini (derived from the accessibility to specific antibodies) we propose a structural model for the type II transporter as shown in Figure 1 [10]. Only limited information on the structure of the type I transporter is available; hydropathy analysis suggested that it spans at least seven times the membrane [10, 11]. Recent radiation inactivation experiments provided evidence that the 'functional complex' of brush border membrane Na/P_i-cotransport system(s) is most likely homomultimeric (3 to 4 units) [30].

'Cell localization' of renal brush border membrane Na/P_i-cotransporters

For both the type I and type II cotransporters, the related mRNA was localized to the renal proximal tubule [28, 31]. Using specific antibodies and cell fractionation techniques it was documented that the two transporters 'comigrate' with brush border membrane marker proteins [27, 28]. Also, immunohistochemical studies provided evidence for exclusive localizations of the type I and type II cotransporter proteins in proximal tubular brush border membranes [27, 28]. Interestingly, the type II transporter has a highly 'dynamic' localization with cell to cell differences in surface expression and with an additional location in intracellular structures (Fig. 2) [28]. In contrast, the type I transporter seems to be 'distributed' more uniformely at the apical cell surface [27]. Thus, on the basis of the above-described functional characteristics and the 'dynamic' cell location, it is assumed that the type II transporter determines the 'overall' properties of renal proximal tubular brush border membrane P_i-transport and its regulatory control.

PTH-dependent inhibition

Previous studies have documented that PTH leads to a reduction in the apparent V_{max} of brush border membrane Na/P_i cotransport [3, 5]. Tissue culture experiments provided evidence for an involvement of protein kinases A and C, and most likely of an endocytic step in PTH-action [6, 7, 33, 34]. In immunoprecipitation experiments it could be shown that the type II transporter is indeed a phosphoprotein [35, H. Murer and J. Biber, unpublished results]. In an attempt to identify potential phosphorylation sites within the type II transporter molecule we have recently expressed it in *Xenopus laevis* oocytes and have 'modulated' Na/P_i-cotransport activity by pharmacological activation of either protein kinase A or C. In apparent agreement with the presence of protein kinase C sites and the lack of protein kinase A consensus sites (rat, NaPi-2), we found a reduction of transport activity by protein kinase C activation [35]. Site directed mutagenesis of individual or of all of the kinase C consensus sites did not prevent this regulation [35]. Therefore, we must conclude that 'cryptic' rather than 'canonical' sites are involved in this regulation or that a 'regulatory' component (subunit) is involved in this kinase mediated regulation of Na/P_i-cotransport. In immunohistochemical studies we recently provided evidence for the involvement of an endocytic mechanism in PTH-dependent control of rat proximal tubular Na/P_i-cotransport (Fig. 2) [32]. Infusion of PTH led to a reduction in the brush border membrane Na/P_i-cotransport rate, paralleled by a decrease in brush border membrane type II transporter content (Western-blot, immunohistochemistry) and by an increased localization in intracellular compartments ('subapical endosomes'; Fig. 2). It will be of interest to define the

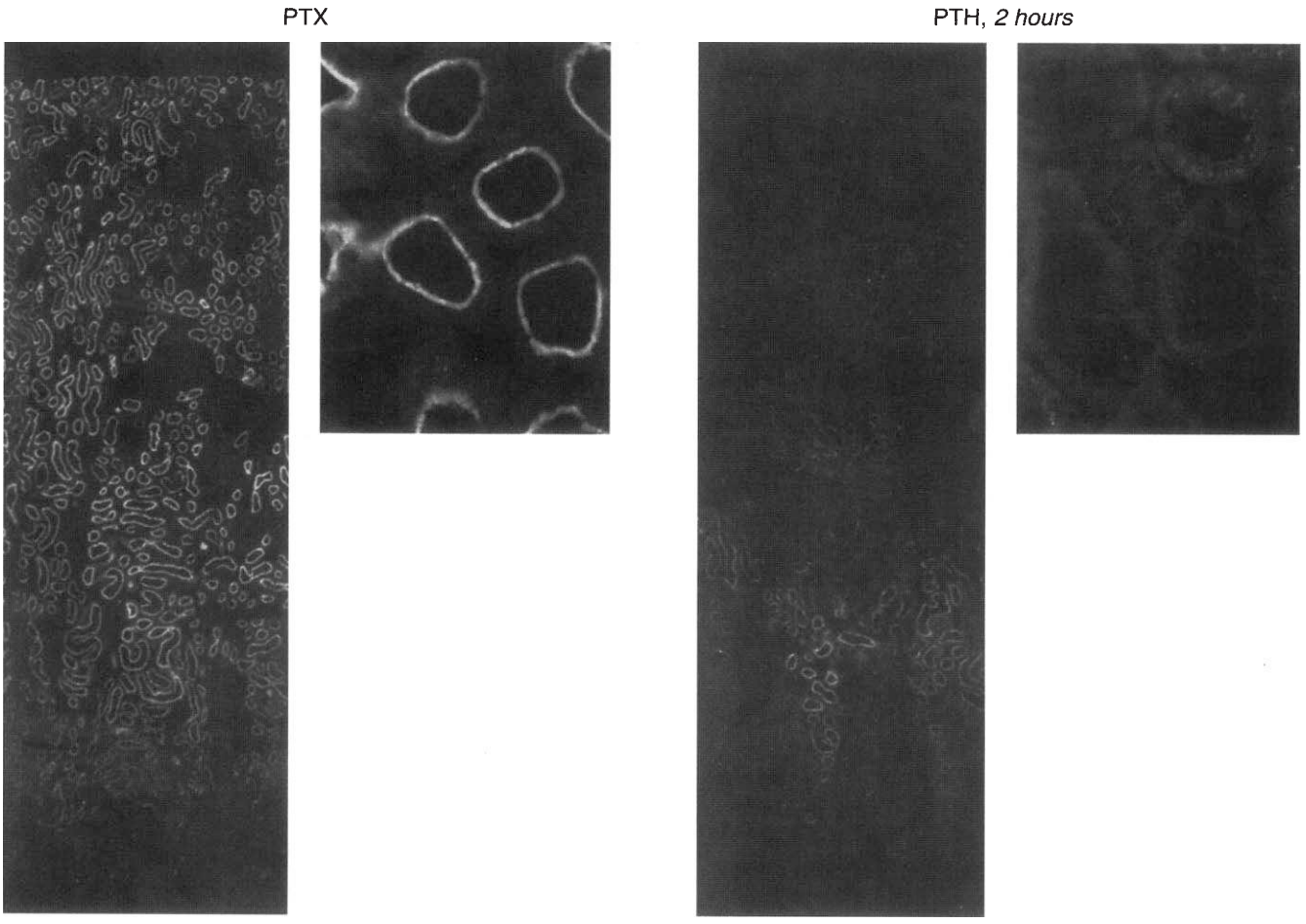

Fig. 2. *Immunohistochemical evidence for an involvement of endocytosis in PTH-dependent inhibition of proximal tubular P_i-reabsorption.* Specific antibody recognition of the type II Na/P_i-cotransporter [28] is visualized. As seen in the figure, within two hours of PTH-injection the type II cotransporter is removed from the brush border membrane; the fluorescence staining at the brush border site is reduced.

signalling pathways (phosphorylations) and the cellular mechanisms leading in PTH-action to the retrieval of the Na/P_i-cotransporters.

Dietary P_i-deprivation

Dietary restriction of P_i is associated with an 'adaptive' increase in proximal tubular P_i-reabsorption and thus in the rate of brush border membrane Na/Pi-cotransport [1, 2, 6, 7, 19, 36–38]. Studies on animals (including also the isolated vesicle technique) and studies on cultured renal epithelial cells have documented that the 'adaptive' response is in part a 'direct' response of the proximal tubular epithelial cells and involves a fast protein synthesis-independent component as well as a slow protein synthesis-dependent component [6, 7, 39]. Recently, using the newly available molecular tools (cDNA probes, antibodies), we have analyzed the effect of altered dietary P_i-supply in thyroparathyroidectomized rats (Fig. 3) [40, 41]. Feeding a low P_i diet increased quickly within two hours the rate of brush border membrane Na/P_i-cotransport; this increase was parallelled by the type II transporter content in the brush border membrane (Western blot; immunohistochemistry). A reverse result was obtained when a high P_i diet was given (for 2 hr) to animals adapted 'chronically' (7 days) to low P_i diets. In this case transport was down-regulated, parallelled by a decrease of the type II cotransporter in the brush border membrane. Feeding a low P_i diet did not alter the specific mRNA content within the two hour time period; however, an increase could be observed at four hours. Re-feeding high P_i-diets to animals 'chronically' adapted to low P_i diets did not lower the specific mRNA content within two to four hours. These studies suggested that adaptation of tubular P_i reabsorption to dietary P_i intake consists of two components: (1) a fast increase in surface expression not related to alterations in mRNA-content; and (2) a 'slow' (adaptive) response requiring changes in mRNA content via transcription and/or stability. Again, it will be the task of future experiments to define the cellular mechanisms involved in these regulatory phenomena.

X-linked hypophosphatemia

A 'direct' involvement of brush border membrane Na/P_i cotransport in the renal P_i leak associated with X-linked hypophosphatemia has been known for many years [8, 38, 42, 43]. In recent studies it could be shown that the brush border membrane type II Na/P_i-cotransporter protein and kidney cortex mRNA content is

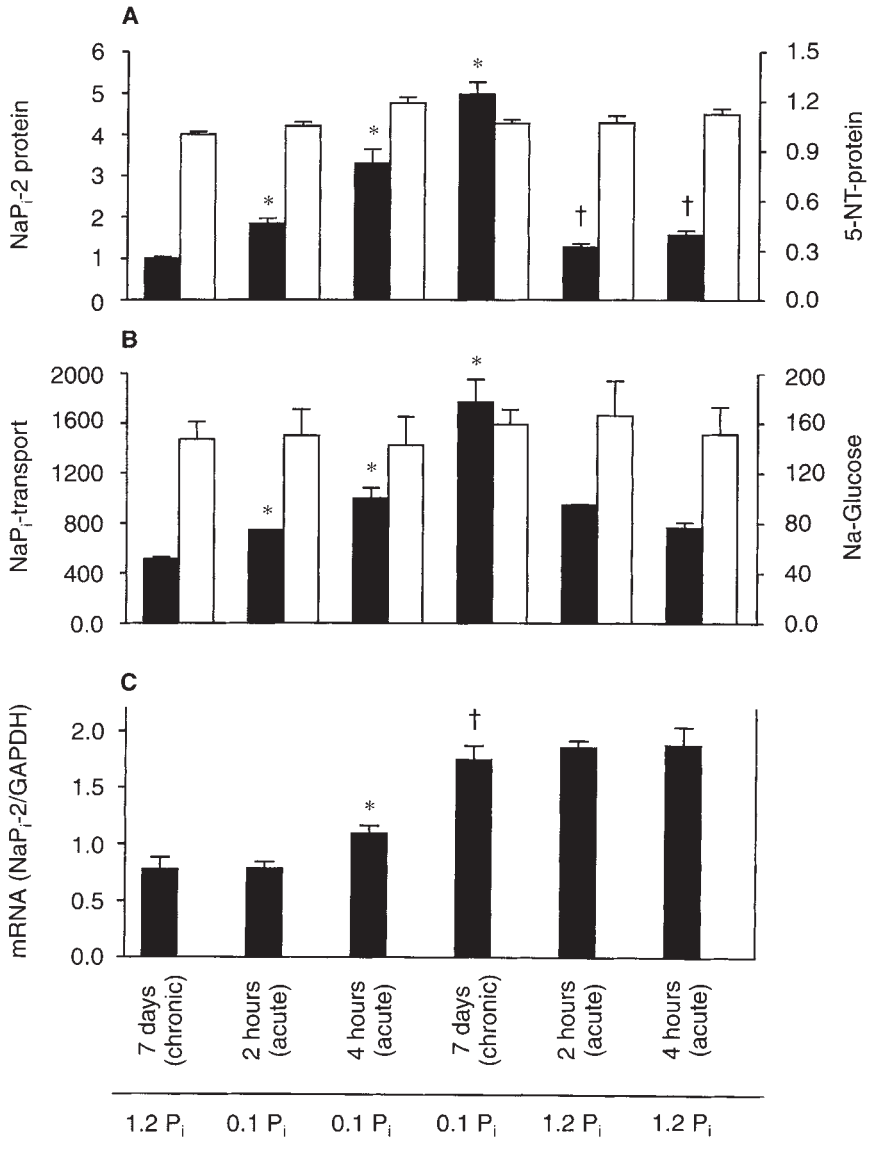

Fig. 3. *P_i-deprivation dependent alterations in (**A**) rat type II transporter (NaPi-2) protein content, (**B**) rat brush border membrane Na/P_i-cotransport activity, and (**C**) specific rat type II transporter mRNA content (NaPi-2).* Rats were fed for seven days with a diet containing high P_i (1.2 P_i) and were exposed for different time periods to low P_i (0.1 P_i). Rat's chronically adapted to low P_i have been re-fed with high P_i for either two or four hours. The data are derived from a recent publication [40]. Symbols in A are: (■) Na/P_i transport; (□) 5-NT-protein. Symbols in B are: (■) Na/P_i transport; (□) Na-glucose.

reduced in parallel with reduced cotransport activity [42]. However, the type II transporter is not on the X-chromosome but on chromosome 5 [44]. Thus, the X-linked factor may control the transcription rate (or mRNA stability) of the type II transporter. On the other hand, the type II transporter is a likely candidate for being involved in autosomal forms of hereditary hypophosphatemic rickets. The type I transporter, located in the human to chromosome 6 [13], seems not to be able to contribute in a major way to the overall renal handling of P_i (see above), and therefore does not seem to be involved in hereditary disorders of renal P_i-handling.

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